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## **Sex Determination and Differentiation in Vertebrates**

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The concept of sex determination and differentiation has been baffling mankind since time immemorial. While Aristotle hypothesized in 335 B.C. that sex was determined by the heat generated during conception, Andreas Vesalius in 1543 A.D illustrated that women were but males, their genitalia turned inside out. In numerous cultures since then, women have been considered as the “default state of men”. However, in the 17<sup>th</sup> century gradually it was discovered that the females produced eggs that transmitted parental traits. Later, in the twentieth century Geddes and Thomson came forward with the hypothesis that constitution, age, nutrition and environment of parents decide the sex determination of the offspring. This environmental view of sex determination was again challenged by the discovery of sex chromosomes by McClung in 1902. Following this, more evidences gradually clinched to suggest the chromosomal concept of sex determination. Numerous experimental studies in vertebrates have now established beyond doubt that sex is determined either by chromosomal factors, environmental influences, or interplay of both, depending on the species/groups.

**Environmental Sex Determination (ESD):** In some cold blooded vertebrates (fishes, reptiles), sex is determined not at the time of fertilization by sex chromosomes, rather, it is determined by the environment in which the early embryonic development takes place.

In various freshwater fishes as well as marine species from temperate or tropical habitats, it has been clearly shown that sex determination and consequent differentiation is influenced by the environmental factors like pH, salinity, social interaction and importantly, temperature (Baroiller & D’Cotta, 2001). In most thermosensitive species (some Atherinids,

Poecilids, Cichlids, a Siluriform), high temperature increases male to female sex ratios and vice-versa. Exceptionally, in European sea bass, *Dicentrarchus labrax* and channel catfish, *Ictalurus punctatus* high temperature induces female sex differentiation. Density and pH also plays a sex determining role in fishes as evidenced in paradise fish *Macropodus opercularis*, where the number of females is directly proportional to the density and in all *Apistogramma* species (cichlids) the proportion of males is higher at an acidic pH (4.5) than at neutral pH (6.5). The complete sex reversal can also be accomplished by exposing gonochoristic fish to exogenous sex steroids during gonadal differentiation. In addition to gonochorism, several types of hermaphroditism are seen in fishes. Social interaction such as disappearance of a male or female from a mixed group causes sex inversion in these species: from male to female (protoandrous, sea anemone fish, *Amphiprion clarkii*), from female to male (protogynous, Red sea fish, *Anthias squamipinnis*) and a few can change sex either way or multiple times (serial sex change, gobiid fish, *Trima okinawae* (Baroiller *et al.*, 1999)

In amphibians, although sex chromosomes are invariably shown responsible for sex determination with either male heterogamety (XY/XX) or female heterogamety (ZW/ZZ), the environmental factor and hormones can override the genotypic mechanism of sex determination and induce sex reversal. The rearing of larvae of salamanders (ZZ/ZW) at high temperatures (30-32 °C) produces opposite effects in two different species. ZZ genotypic males of Algerian ribbed newt, *Pleurodeles poireti* become phenotypic females, whereas ZW genotypic females of Spanish ribbed newt, *Pleurodeles waltl* become phenotypic males. In another newt *Triturus cristatus* (XX/XY), more males than females, including some XX neomales are produced at higher temperature, and vice-versa at lower temperature. Also, in wood frog, *Rana sylvatica*, sex reversal of all females occurs when larvae are reared at high temperatures (32±2 °C). However, all the above said studies have been conducted at extreme temperature which is outside of the range normally experienced by these species, and

therefore, these effects probably do not occur under natural conditions. An exception is common frog, *Rana temporaria*, where temperature of only 20 °C masculinizes the females. Nevertheless, the effect of temperature in all these cases is limited to a particular stage i.e., before the first sign of histological differentiation of gonads. Social interaction, in addition to temperature, also causes sex reversal. In case of common reed frog, *Hyperolius viridiflavus* low male density induces sex reversal in females (Eggert, 2004). Perplexing response to hormone treatment is observed in different species of XX/XY ranids and hylid frogs : masculinization by testosterone and feminization by estradiol (Northern leopard frog, *Rana pipiens*), masculinization by testosterone but impervious to estradiol (black spotted frog, *Rana nigromaculata* and Japanese brown frog, *Rana japonica*), feminization by estradiol but non responsive to testosterone (Japanese tree frog, *Hyla japonica*), and refractory to both, testosterone as well as estradiol (yellow bellied toad, *Bombina variegata* and Oriental fire bellied toad, *Bombina orientalis*). Other amphibians that have heterogametic females (ZW), e.g. *Bufo*, *Xenopus* and some urodeles are feminized by estradiol but not masculinized by testosterone. A bizarre exception in this case is shown by racophorid frog, *Buergeria buergeri*, where ZW tadpoles are masculinized by either estradiol or testosterone treatment but ZZ tadpoles are not affected appreciably by either hormone (Wallace *et al.*, 1999).

Reptiles exhibit extraordinary variability in patterns of sex determination among vertebrates. Male heterogamety (XY or XXY) is known in turtles, female heterogamety (ZW, ZZW or ZWW) is known in snakes and both are known in lizards. Even in the absence of any gross heteromorphy in the sex chromosome, genotypic mode of sex determination is seen in some species. However, the crucial role of environment in sex determination was seen long back in the year 1966 in lizard *Agama agama* where incubation of eggs at different temperature changed the sex ratios of hatchling. Later, environment (temperature)-dependent sex determination was seen widespread in reptiles. Temperature-dependent sex determination

(TSD) is invariably seen in all tuatara and crocodiles, most tortoises, turtles and terrapins, and in some lizards (Pieau C, 1996). Generally, among most chelonians, incubation of eggs below transition range temperature (TRT) induces masculinization, above TRT leads to feminization, and at TRT both males and females or even intersex individuals are produced. On the contrary, in alligators, lizards and tuatara males are produced at high temperature and females at low temperature. However, exceptions to these trends are seen in crocodiles and a few turtles like snapping turtle, mud turtle, alligator snapping turtle etc., where eggs incubated below or above TRT develop into females and males are produced at TRT. In all TSD species there is a well defined thermosensitive period (TSP) when temperature affects sex determination and after this the embryos become refractory to temperature changes.

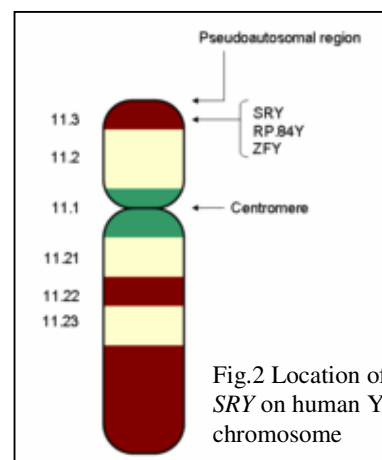
Temperature influences sex determination by modulating the activity of steroidogenic enzymes and thereby affecting the hormonal environment of the embryo inducing either masculinization or feminization. In freshwater turtle, *Emys orbicularis*, the aromatase activity responsible for aromatization of testosterone into estradiol remains low at male producing temperature (25 °C) and high at feminizing temperature (30 °C). Moreover, exogenous sex steroids can override the effect of temperature on sex determination in TSD species. Earlier, it was thought that the hormone induced sex reversal is unilateral towards the female sex, since administration of estradiol or testosterone induces embryos to develop as females even if eggs are incubated at male sensitive temperature. However, in case of red-eared slider turtles, *Trachemys scripta*, instead of testosterone, administration of dihydrotestosterone (DHT, a non aromatizable androgen) during TSP altered the unilateral pattern of sex reversal and induces masculinization. Identical result is observed when aromatase inhibitor (AI) is applied to eggs incubated at female sensitive temperature. Although sex is genetically determined in the Indian garden lizard, *Calotes versicolor*, exogenous androgen induces sex reversal. DHT is found to be effective only between stages of initialization of genital ridge

and formation of sex cords (Ganesh *et al.*, 1995). Previous experiences of unilateral sex reversal towards females after administration of testosterone to eggs incubated even at male sensitive temperature might be due to the aromatization of testosterone into estradiol, the female sex steroid.

**Chromosomal Sex Determination (CSD):** Before the karyotyping of human chromosomes, it was considered that sex is determined by the number of X chromosomes present in an individual. It was observed that in drosophila, males had single X chromosome (XY or XO) and the presence of 2 or more X chromosomes (XX, XXX, or XXY) always conferred female phenotype. It was thus considered that the Y chromosome was a null chromosome. However, in 1966, a landmark observation was made by Jacobs and Ross. They described two sisters who had female external genitalia but 46 XY karyotype in which the Y chromosome consisted only of its long arm. It seemed therefore, that the testicular determining region of the Y chromosome normally resides in its short arm. This was confirmed by high resolution banding studies of an XX male which revealed that some material from the short arm of Y chromosome had been translocated to one of his X chromosomes. With advanced techniques, it became apparent that sex determining gene on short arm of Y chromosome is responsible for the development of testis in mammals. Also, testicular development is seen impaired in several clinical syndromes resulting from autosomal deletions or mutations. Now autosomal genes such as *SOX 9*, *WNT 4*, *SF1*, *DMRT 1* etc are shown to play a crucial role in the downstream events of sex differentiation initiated by *SRY* (Fig 1).

***SRY*** (Sex determining region on Y chromosome): *SRY* gene is located near the tip of the short arm of Y chromosome that encodes a transcription factor of 204 amino acids. The central 79 amino acids encode the HMG (high mobility group) box. In the entire *SRY* protein, only HMG domain shows sequence conservation. HMG box functions as DNA-

binding, and DNA-bending domain and also has two nuclear localization signals essential for translocation of protein into the nucleus. However, the non conserved regions outside the HMG box are also essential for SRY function, since a truncated SRY protein lacking the carboxy end is unable to induce male development in XX transgenic mice. As an architectural transcription factor, SRY unwinds the DNA and bends it to almost 80 degree thereby, bringing the other distantly bound transcription factors in close contact. The exact binding site of SRY on the DNA and the mechanism



through which SRY acts is still not resolved (Harley *et al.*, 2003). Nevertheless, the ultimate function of SRY is the up regulation of SOX 9. It is interesting to note that *SRY* is absent in monotremes (egg laying mammals: platypus and echidna) and in non-mammalian vertebrates.

**SOX 9** (SRY-related high-mobility group box 9): *SOX 9* present on chromosome 17 in human is a highly conserved autosomal gene responsible for testicular differentiation. Like *SRY*, *SOX 9* encodes a transcription factor that also contains a HMG box and a transactivation domain in the C-terminus. *SOX 9* was discovered in an investigation of campomelic dysplasia (CD), a disease involving bone and cartilage disorder. XY patients with this disease developed as phenotypic females. Mutational analysis revealed that absence of *SOX 9* is responsible for CD as well as XY sex reversal.

In mouse, at 10.5 days post conception (dpc), just before or around the same time as *Sry* transcripts are first detected, *Sox 9* is expressed at low levels in the developing gonads of both sexes. By 11.5 dpc, *Sox 9* is robustly expressed in the XY gonads and is completely absent from XX gonads. Although its expression is up regulated by *Sry* expression, *Sox 9* remains active in embryonic testis long after *Sry* expression has ceased. SOX 9 along with

other transcription factors activate the expression of *Amh* gene (anti Müllerian hormone gene). The binding of SOX 9 HMG box bends the DNA which bring SF1 and GATA 4 in close proximity to each other and along with WT1 and HSP 70 form a tightly associated protein complex that activates transcription of the *Amh* gene. The complete absence of AMH transcripts is seen in XY mice mutant for HMG box (DNA binding domain) of *Sox 9* gene suggesting that *Sox 9* is required for AMH expression.

Although experimental evidences in non mammalian vertebrates show that *Sox 9* has a conserved role in sex determination, it is important to mention here that its expression in alligator and chicken begins well after pre Sertoli cell differentiation and AMH expression. It seems that *Sox 9*, in non mammalian vertebrates may be involved in Sertoli cell organization, rather than early testicular determination (Pask & Graves, 1999).

**SF1** (Steroidogenic factor 1): *SF1* gene transcribes a protein, otherwise known as Ad4BP, belonging to the orphan nuclear receptor family. Initially, SF1 was described to regulate the production of cytochrome P-450 steroid hydroxylase enzymes that are necessary for synthesis of steroids, and thus, are expressed in many steroidogenic tissues, including adrenal gland, ovary and Leydig cells of the testis. Subsequently, SF1 transcripts were detected in the mouse urogenital ridge even at the stage of the indifferent gonad (9-12 dpc) and mutation in *Sf1* gene was shown to cause complete dysgenesis of gonad in both sexes. This suggests its role in early formation of the indifferent gonad and, thereby, *Sf1* is placed upstream of *Sry* in sex determination pathway. However, *Sf1* also plays an important role in the downstream testicular differentiation pathway initiated by *Sry*. *Sf1* activates testicular differentiation by influencing both, Leydig and Sertoli cells. SF1 in Leydig cells regulates steroid biosynthesis and in Sertoli cells it binds to *Amh* promoter region and activates the expression of AMH in collaboration with other transcriptional factors. The importance of *SF1*, located on

chromosome 9, for testis development and *AMH* regulation in humans is demonstrated by XY patient heterozygous for *SF1* where the individual has malformed fibrous gonads and retains fully developed Müllerian duct structures (Achermann *et al.*, 1999).

**WT1** (Wilms' tumor 1): The *WT1* gene first came into focus in patients with Wilms's tumor where mutation of this gene led to embryonic kidney tumor. Later, mutation in this gene was seen associated with the disruption of bipotential gonadal development. The presence of WT1 transcripts in developing gonads substantiates its role in early bipotential gonadogenesis.

*WT1* encodes variant transcripts by alternative splicing, alternative translation start sites, and RNA editing. These variants give rise to different zinc finger DNA-binding protein isoforms that fall under two categories (designated as +KTS and – KTS) depending on the presence or absence of three amino acids (KTS, lysine-threonine-serine) between two zinc fingers. Experimental evidences in mice show that the -KTS isoform is responsible for development of undifferentiated gonad. WT1 +KTS isoform plays an important role in testicular differentiation possibly by regulating *Sry* expression, since potential WT1 binding sites are present upstream of both mouse and human *SRY*. Further more, mutation in *WT1* gene causes persistence of Müllerian duct in males. In recent years, WT1-KTS isoform is shown to physically interact with SF1 and synergistically up regulate AMH expression.

**GATA4/FOG2:** GATA4, a member of the GATA family of transcription factors, contain a zinc finger DNA-binding domain that binds to the consensus sequence WGATAR in the 5'-flanking region of target genes. In mice, it is detected as early as embryonic day 11.5 in the somatic cells of primitive gonads of both sexes and therefore, GATA4 in conjunction with other transcription factors play role in development of bipotential gonad (Viger *et al.*, 1998). The GATA4 expression remains maintained in Sertoli cells throughout embryonic development, whereas it is down regulated shortly after differentiation of ovary on embryonic



day 13.5 in mice. The presence of a conserved GATA4 sequence in the promoter site of *Amh* gene further substantiates its role in inducing masculinization. Mutation of GATA4 in XY mice embryos lead to ovarian development, indicating its possible role in *Sry* transcription. During urogenital development, GATA4 is expressed with FOG 2 (friend of GATA) in the same somatic cells of gonad, implicating their close interaction in bipotential gonad development (Swain, 2006). Furthermore, mouse fetuses homozygous for a null allele of *Fog2* exhibit abnormalities in male sex differentiation.

***DMRT 1*** (double sex-and mab-3 related transcription factor): *DMRT 1* belongs to the family of genes that encode proteins containing DM-domain, a novel DNA-binding motif. It is one of the most conserved genes in sex determination, since its presence is observed across the phyla from invertebrate to vertebrate. *DMRT 1* that maps to the distal arm of chromosome 9 in humans is homologous to the double sex (*dsx*) in *Drosophila melanogaster* and mab-3 in *Caenorhabditis elegans* which are involved in sex determination in their respective species. Although its expression coincides with *Sry/Sox 9*, maximum expression of *DMRT 1* is seen in Sertoli cells during post natal testis development.

***DAX 1*** (DSS-AHC critical region on X chromosome, 1): *DAX 1* maps on short arm of X chromosome and encodes for a protein that belongs to the orphan nuclear receptor family. Duplication of this gene causes male-female sex reversal (dosage-sensitive sex reversal, DSS), whereas its deletion results in adrenal hypoplasia congenita (AHC). *DAX 1* is initially expressed in genital ridges of both sexes. Its expression persists in case of developing ovary while it is drastically down regulated with testis differentiation. *DAX 1* suppresses testis differentiation at two levels: one, by inhibiting SF1-induced *SRY* expression in a bipotential gonad, and two, by repressing synergistic action of SF1 and WT1 and thereby, suppressing

downstream genes, *e.g.* *Amh* and other steroidogenic genes. Thus, it is considered as ovary-determining gene.

**WNT 4:** *WNT 4* is another important ovary determining gene. Like *DAX 1*, its expression is turned off with the differentiation of testis. *WNT 4* knock out XX mice shows masculinization as ovarian differentiation ceases and its cells express testis-specific markers, including *Amh* and testosterone producing enzymes (Vainio *et al.*, 1999). This suggests that *WNT 4* is obligatory gene for female sex differentiation.

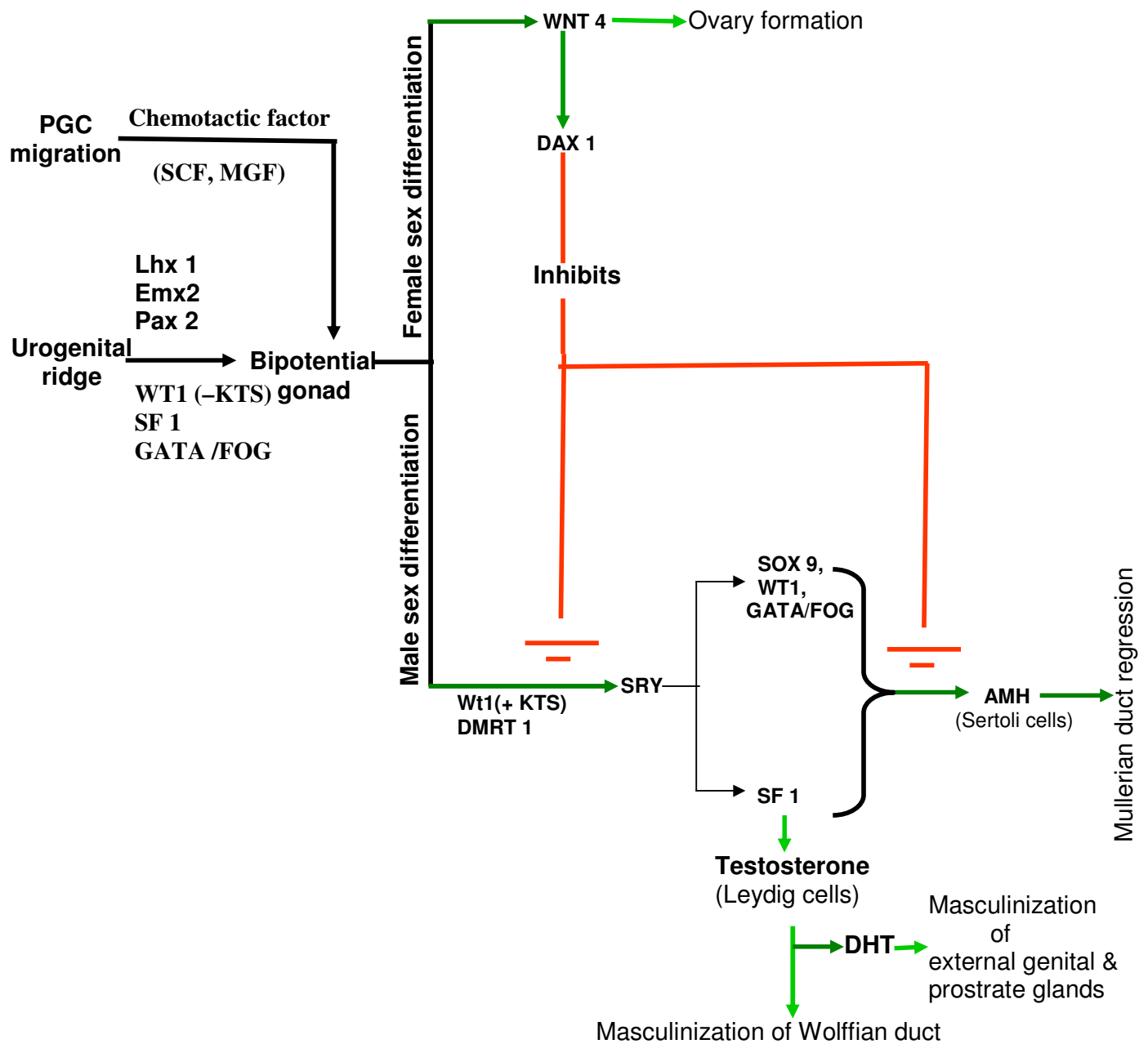
In marsupials too, the control of testis determination is vested in the Y chromosome, though it is the smallest of any mammals (Graves and Shetty, 2001). However, the number of X chromosome plays a critical role in other aspects of sex differentiation (Table.1). Single X chromosome in XO animals leads to the development of empty scrotum (without testis), whereas scrotum fails to develop in XXY animals having testis. In fact, the presence of two X chromosomes leads to the development of pouch and mammary glands in lieu of the scrotum (Manolakou *et al.*, 2006). Thus, marsupials are different from other mammals with regard to the accessory sex organ differentiation since the formation of scrotum, pouch and mammary glands in marsupials are dependent on genes present on X chromosomes rather than on gonadal hormones as in eutherian (Pask and Benfree, 2001).

**Table 1: X-linked secondary sexual differentiation in marsupials**

	<b>Y chromosome (testis)</b>	<b>Absence of Y chromosome (no testis)</b>
<b>Single X chromosome (scrotum)</b>	<b>XY</b> (males with scrotum and testis)	<b>XO</b> (males with empty scrotum)
<b>Double X chromosome (pouch and mammary glands)</b>	<b>XXY</b> (males with testis, pouch and mammary gland)	<b>XX</b> (females with pouch and mammary glands)

However, the monotremes are unusual because they have multiple set of sex chromosomes rather than the single pair usually found in marsupials and eutherians. Recent report demonstrates the presence of 10 sex chromosomes in platypus arranged as X1Y1X2Y2X3Y3X4Y4X5Y5 (Rens *et al.*, 2004). Interestingly, genes present on X chromosome situated at one end of the 'sex chromosome chain' are orthologous with those on the human-X chromosome, while genes on chromosome situated at the other end of the chain are homologous with those on the bird-Z chromosome. Hence, the platypus provides an important link between the chromosomal sex determination in mammals and birds.

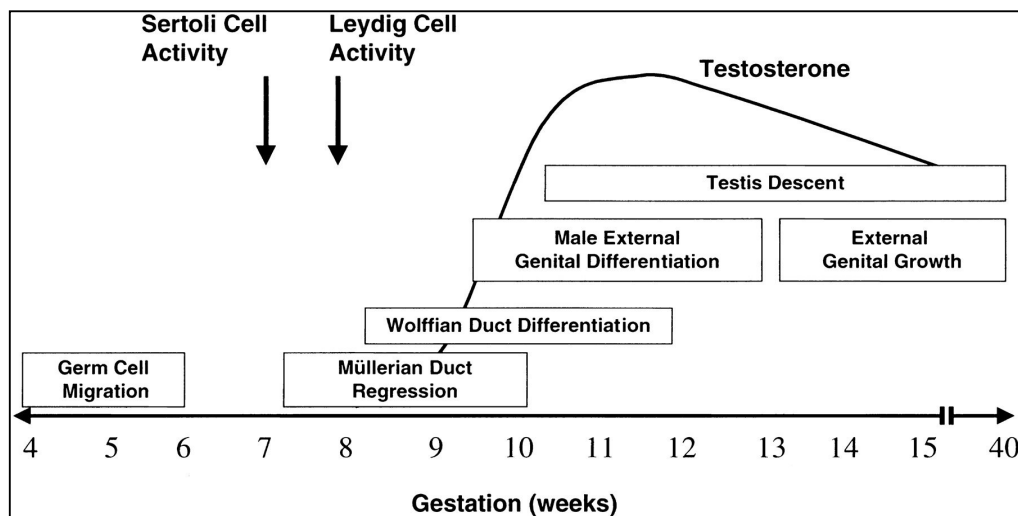
Birds have distinguished sex chromosomes, except ratitae (e.g. ostrich, emu) which have karyologically indistinguishable or slightly differentiated sex chromosomes (Schartl, 2004). The sex chromosomes have been designated as Z and W chromosome. Unlike mammals, males are homogametic (ZZ) while females are heterogametic (ZW). However, whether W chromosome carrying dominant female determining genes (*ASW*: avian sex specific W-linked, and *FET1*: female expressed transcript 1) or the Z chromosome carrying a dosage-dependent male determinant gene (*Dmrt1*), or both features determine the sex is not yet clear. Nevertheless, many of the downstream sex determining genes (*Sox9*, *Amh*, *Dmrt1*, *Sf1*, *Wt1* and *Dax 1*) are reported in birds though the order of their expression in developing gonad may be different from that reported in mammals. Chromosomal sex determination is prevalent also in amphibians, though environmental and hormonal factors are reported to cause sex reversal. Although in most of the fishes and reptiles sex is determined by environmental factors, downstream sex determinant genes are reported. However, their interaction, temporal expression and the initial trigger does not follow any taxonomic scheme.



**Fig. 1.** Schematic representation of chromosomal sex determination and differentiation in mammalian model

## Sex Differentiation:

The sexually indifferent bipotential gonad develops from the ventromedial surface of the mesonephros near the kidneys at around 4 weeks in the human fetus and 9.5 days post-coitum (dpc) in the mouse. The somatic cells of gonad are derived from the mesonephros and coelomic epithelium that covers the coelomic surface of the gonadal ridge. These cells proliferate in the gonadal primordia and form the sex cords. Several factors such as Lhx1 (LIM Homeobox gene1), Emx2 (homolog of empty spiracles homeobox gene 2), Pax 2 (paired box gene 2), WT1 and SF1 are involved in cell proliferation and development of the



**Fig 3. Embryologic events in male sex differentiation depicted in temporal fashion. The line depicts the increase in fetal serum testosterone concentrations. The word activity refers indirectly to the action of AMH in causing Müllerian duct regression and androgens to induce male sex differentiation (Hughes, 2001)**

bipotential gonad. The gonad is subsequently colonized by primordial germ cells (PGC) that originate from epiblast-derived cells present in the yolk sac near the base of allantois and migrate through the hindgut to invade the indifferent gonad. Germ cell migration is under the influence of stem cell factor (SCF), mast cell growth factor (MGF), and extra cellular matrix proteins like fibronectin and laminin (Bendel-Stenzel *et al.*, 1998). During migration the germ cells proliferate but do not differentiate. The primordial germ cells are distinguishable

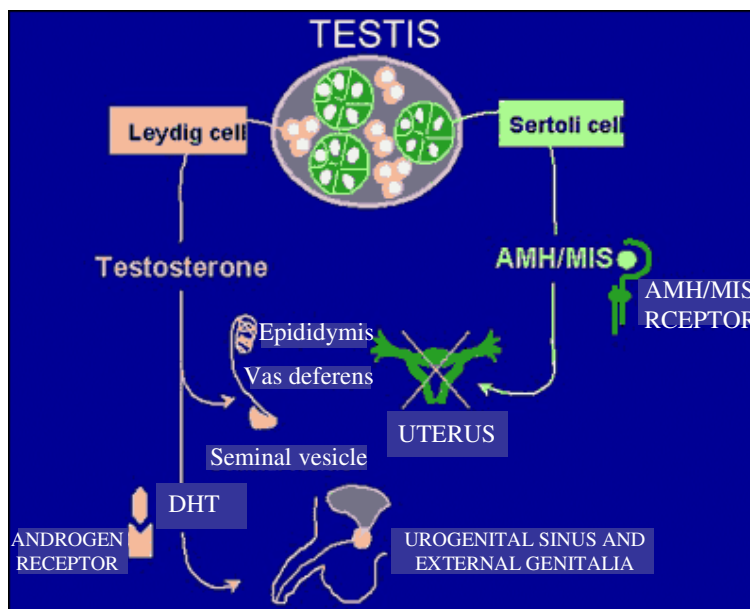
from the other cell type because of their large size and large round nuclei. Histologically, they are identified by high alkaline phosphatase activity and glycogen. PGCs along with the somatic cells form the “gonadal ridge”. The formation of gonadal ridge is completed within 5 to 6 week of gestation in human embryos. No sexual difference can be observed in the gonads until the 6<sup>th</sup> week of embryonic life in humans and 11.5 dpc in mice.

### Male Sex Differentiation

In XY fetus of 6-7 weeks, the first sign of testis differentiation is seen with the aggregation of the pre Sertoli cell (derived from mesonephros) around the germ cell now called gonocytes, to form the testicular cords. These cords lose contact with the surface epithelium and become separated from it by a thick extra cellular matrix, the tunica albuginea. By the end of 9 week, the mesenchyme that separates the seminiferous cords gives rise to interstitial cells. Later these are differentiated into steroid secreting Leydig cells. Although differentiation of Leydig cells in the initial phase is independent of gonadotropin action, its proliferation and differentiation in the first and second trimesters of the fetal life depends on placental hCG, and thereafter, controlled by fetal pituitary LH (Josso *et al.*, 2005).

The masculinization of the genital tract starts with the regression of Müllerian duct under the influence of anti Müllerian hormone (AMH) secreted from Sertoli cells. Shortly after the Müllerian duct regression, the portion of Wolffian duct adjacent to testis is differentiated into epididymis, the central portion becomes vas deferens, and the distal end of the duct near the urogenital sinus develops into seminal vesicle. The prostate gland develops as a series of outgrowths from the urogenital sinus (Fig. 4). The virilization of the Wolffian duct is under the control of testosterone as the phenomenon is seen inhibited after the administration of anti-androgen or testosterone antibody at the critical period of sex differentiation (Elger *et al.*, 1970).

Under the influence of androgen, the male external genitalia start differentiating around the 9<sup>th</sup> week of gestation in case of human. The genital tubercle elongates to form the phallus and scrotum, and the urethral folds fuse over the urethral groove. Although testosterone plays primary role in differentiation of Wolffian duct into epididymis, vas deferens and seminal vesicle, there is evidence that it is not the active masculinizing hormone in certain tissues. It is dihydrotestosterone (DHT) that masculinizes urogenital sinus and genital tubercle into prostrate and penis, respectively (Fig. 4). The role of DHT came into light when XY children lacking a



**Fig 4: Role of different hormones in male sex differentiation (Josso *et al.*, 2005)**

functional gene for 5- $\alpha$ -reductase, the enzyme that converts testosterone to DHT, were reported to have a blind vaginal pouch and enlarged clitoris. However, they have male internal anatomy: developed testis, epididymis, vas deferens and seminal vesicle (Thigpen, 1992). Administration of 5- $\alpha$ -reductase inhibitor in rats is shown to severely affect the masculinization of external genitalia.

**Testicular Descent:** The factors controlling testicular descent has been the subject of much controversy. Previously, AMH was considered to be associated with testicular descent as decreased AMH level is usually seen in cryptorchid patients. Later, testicular dysgenesis was cited as the cause of cryptorchidism. Recently, Insulin like factor 3 (INSL3) secreted by fetal Leydig cells and belonging to the insulin/relaxin super family has been shown to be involved

in the gubernaculum development in INSL3 mutant mice ( Nef & Parada ,1999). Mutation of this gene has been detected in cryptorchid patients. Moreover, androgens mediate the disappearance of the cranial suspensory ligament (van der Schoot & Elger, 1992) and are required for the inguinoscrotal phase of testicular descent.

#### Female Sex Differentiation:

In females, the primary sex cords undergo degeneration and a new set of sex cords is then produced by the epithelium. These sex cords reside in the periphery and hence, are called cortical sex cords. The primordial germ cells proliferate by mitosis and give rise to oogonia that enter into meiosis by 10<sup>th</sup> week in human fetus and form the oocyte. The cortical cords surround the oocytes and form granulosa layer. The formation of primordial follicle, oocyte surrounded by a single layer of flattened granulosa cells, commences around 16<sup>th</sup> week of gestation. This is the pool from where Graafian follicles are formed (primordial-primary-secondary-tertiary/Graafian) by week 23-24 in human fetus. Oocytes proceed to the diplotene stage and remain arrested till the time of puberty.

Female differentiation of the internal genital tract is characterized by the regression of the Wolffian duct that disappears at 90 days of human fetal development. Vestiges remain in the form of Gartner canals and Rosenmüller organs. The Müllerian duct differentiates into oviducts, uterus, cervix, and upper vagina. Although the role of estrogen in female fetal development was obscure earlier, in recent years it is seen indispensable for Müllerian duct differentiation using null mouse model. The secretion of estradiol in XX gonads starts at the same time when testosterone synthesis begins in XY gonads (George & Wilson, 1978). Nonetheless, the female sex differentiation in eutherian mammals takes place in a sea of factors from the placenta, maternal circulation and the fetal gonads. Therefore, one should be cautious while describing the role of any factor in female sex differentiation.



### Anomalies of Sex Determination and Differentiation:

The anomalies at any step of sex determination/differentiation leads to disorders. The alterations might be chromosomal, gonadal or phenotypical. The opportunities for mishaps are considerably high in males than females since the male sex development is very active and highly complicated process. The chromosomal abnormalities occur due to numeric changes in the sex chromosomes. For example, only one X chromosome is present in females with Turner's syndrome (XO). The bilateral streak gonads and incomplete development of sexual characteristics with primary amenorrhea at puberty is reported in these females. On the other hand, an extra X chromosome in males due to non-disjunction of X chromosomes during oogenesis leads to Klinefelter's syndrome (XXY). Males with this syndrome have small testis, azoospermia resulting in infertility, low concentrations of testosterone, high levels of gonadotropins and poor virilization. Similarly, triple X (XXX) syndrome is reported in females.

In case of gonadal anomalies, genetically females (XX) generally have male external genitalia. This results either due to defects in sex determining genes on autosomes or linking of a testis determining gene, otherwise present on Y chromosome, with autosome. These females have either cryptorchid testis or atleast one ovo-testis. The gonadal dysgenesis is also true for 46 XY males with defects in Y chromosome or sex determining genes on autosomes.

Phenotypical anomalies arise due to imbalance in hormone milieu or its responding machinery during development. In this case, the chromosomal sex and the gonadal sex match up, but the ambiguity in external phenotype results in pseudo-hermaphroditism. Female (XX) pseudo-hermaphroditism occurs as a result of excess androgens during embryo development. The masculinization of external genitalia is prevalent, though these females have normal ovaries. In case of XY males, pseudo-hermaphroditism might arise due to androgen

insensitivity syndrome of embryo. They develop feminine characteristics due to lack of masculinization.

## Conclusion

The basic pattern of gonadogenesis though remains similar, the factors determining sex of embryo vary considerably among vertebrates. Sex is primarily determined by either environmental factors or chromosomal mechanisms. In fishes, temperature is the most important factor in sex determination. Generally, high temperature favours male to female sex ratio in thermosensitive fishes. The temperature-dependent sex determination is also prevalent in living reptiles except ophidians (snakes) where distinct sex chromosomes are present (ZZ/ZW). Intriguingly, sex in amphibians is primarily determined by chromosomal mechanism with heterogamety in male (XY/XX) as well as female (ZW/ZZ) depending on species, though sex reversal is reported at unusually high temperatures. Nonetheless, in these thermosensitive amphibians, sex at the ambient temperature is predominantly determined by sex chromosomes. The genetic mechanism by which sex is determined in birds remains to be elucidated. Further, Z and W chromosomes do not share homology with the mammalian X and Y chromosome. Possibly, gene linked to W chromosome determines the ovarian differentiation or Z-linked gene (dosage-sensitive) play a critical role in establishing the male sex. Unlike birds, males are heterogametic (XY), while females are homogametic (XX) in mammals. *SRY* and *SOX 9* are the major testis determining genes. *WT1* and *DMRT1* upregulate the expression of *SRY* that in turn induces the downstream gene *Sox 9*. This gene located on human autosome 17 encodes a transcription factor that along with other factors, *SF1* and *WT1* activate the expression of *AMH* gene. *AMH* induces the regression of the Müllerian duct in males. In addition to promoting *AMH* production by Sertoli cells, *SF1* also regulates the androgen biosynthesis in Leydig cells. Like males, female sex is determined by an active process rather than by default as believed earlier. *DAX1* and *WNT4* are now known

to play a pivotal role. DAX1 represses the synergistic action of SF1 and WT1 on AMH expression and also downregulates the other steroidogenic genes. Like the importance of *SRY* for testis differentiation in males, *WNT 4* is assigned as the ovary determining gene in females. Subsequent to formation of testis or ovary from the bipotential gonads, secondary sex determination commences in which hormones play a crucial role. The virilization of Wolffian duct into epididymis, vas deferens and seminal vesicle takes place under the influence of testosterone. However, DHT, and not the testosterone, is responsible for the masculinization of male external genitalia. Further, the testicular descent into the scrotal sacs is regulated by INSL3 and androgen. The knowledge on hormonal regulation of female sex differentiation is rudimentary, however, the indispensable role of estrogen is implicated in the stabilization and differentiation of Müllerian duct into female genital tract. Unlike eutherians, the sex differentiation in marsupials is independent of hormonal control.

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